Mycopathologia (2006) 162: 215–223
DOI 10.1007/s11046-006-0055-4

Ochratoxins: A global perspective

Paul Bayman[1] & James L. Baker[2]
[1]*Depto. de Biología, Universidad de Puerto Rico-Río Piedras, PO Box 23360, San Juan, PR, 00931 , USA;*
[2]*USDA ARS Western Regional Research Center, 800 Buchanan St., Albany, CA , 94710 , USA*

Abstract

Ochratoxins have been overshadowed by better-known mycotoxins, but they are gaining importance. Here we consider ochratoxins in the context of aflatoxins, which are better understood than ochratoxins on many levels. We review recent work on taxonomic distribution, contamination of commodities, biosynthesis, toxicity and regulatory aspects of ochratoxins. We focus on ochratoxins in coffee, since coffee is becoming a key commodity in ochratoxin research and regulation.

Key words: *Aspergillus*, coffee, food safety, mycotoxin, ochratoxin, *Penicillium*

What are ochratoxins?

Ochratoxins are fungal secondary metabolites produced by several species of *Aspergillus* and *Penicillium*. They consist of an isocoumarin moiety and a phenylalanine moiety linked by an amide bond [1]. Ochratoxin A (OTA) is chlorinated, which is unusual for natural products (Figure 1). Ochratoxin B, which is not chlorinated, and ochratoxin C, the ethyl ester of OTA, are less toxic and less common [1]. Most studies on ochratoxins have therefore focused on OTA.

Present and past importance of ochratoxins

In January 2006, the owner of a major Italian grain mill was arrested and charged with importing 58,000 tonnes of Canadian wheat contaminated with 15 μg/kg OTA [2]. The wheat was milled and sold to food processors. Consumers and food processors were disturbed by the news, but human exposure to ochratoxins is nothing new. There is circumstantial evidence for involvement of mycotoxins, including ochratoxins, in historical events, though the evidence for ochratoxins is not as conclusive as for ergot alkaloids.

Mortality rates decreased in Britain and France in the 1750s. The decrease is at least partly attributed to improvements in nutrition and health care, but there is also a mycotoxin theory. Potato consumption increased around this time. Potatoes are less susceptible to mycotoxin contamination than the wheat, barley and rye they replaced – all of which are susceptible to OTA contamination, especially under the poor storage conditions of that era [1, 3]. Also, mass mortality events occurred in years when the weather was conducive to mycotoxin formation [1].

Several archeologists who opened ancient Egyptian tombs died suddenly and mysteriously of unexplained causes. It has been suggested that the cause of death was acute renal failure caused by inhalation of spores containing ochratoxins [4]. There are documented cases of acute renal failure following exposure to ochratoxin-containing spores, but no direct evidence for the mysterious death theory [4].

Which fungal species produce ochratoxins *in vitro*?

Ochratoxin A was first isolated from *A. ochraceus* (hence its name) in 1965, in South Africa [5]. Its

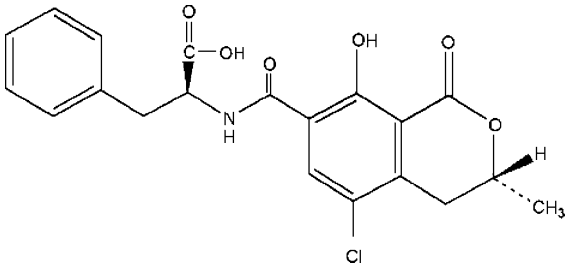

Figure 1. Ochratoxin A.

isolation was part of a large-scale screening for aflatoxins, during the 'mycotoxin gold rush' that followed the discovery of aflatoxins (see [6]).

More *Aspergillus* species were shown to produce ochratoxins in subsequent studies: *A. melleus* and *A. sulphureus*, [7]; *A. alliaceus*, *A. sclerotiorum*, and (reported but not clearly above the limit of detection) *A. ostianus* and *A. petrakii* [8–10]; *A. albertensis* and *A. auricomus* [11]; *Neopetromyces** *muricatus* [12]; and *A. lanosus* [13]. All these species were originally grouped in *Aspergillus* section *Circumdati*, but *A. alliaceus*, *A. lanosus* and *A. albertensis* are now placed in section *Flavi* [14].

Aspergillus ochraceus was polyphyletic as traditionally defined [14–16]. It was recently split into several species, some of which include OTA-producing isolates: *Aspergillus cretensis, A. flocculosus, A. pseudoelegans, A. roseoglobulosus, A. westerdijkiae*, as well as *A. ochraceus sensu strictu* [17].

Ochratoxin A production has also been found in *A. glaucus* (section *Aspergillus*) (see [11]). Later, ochratoxin production was found in *A. niger* var. *niger* [18] and subsequently in other species of *Aspergillus* section *Nigri*: *A. awamori* and *A. carbonarius* [19]; and recently, in two newly described species from coffee, *A. lacticoffeatus* and *A. sclerotioniger* [20]. OTA production in *A. niger* is alarming because this species is used in fermentations for food and enzyme production; industrial strains should be tested for OTA production [21].

Other *Aspergillus* species have been reported to produce OTA, but have been reduced to synomy with the species listed here; for example, *A. alutaceus* was reported to produce OTA [22] but is synonomous with *A. ochraceus* [23]. Reports of OTA production by *A. auricomus*, *A. elegans*, *A. foetidus* and *A. wentii* were apparently based on misidentified isolates [17]. Other species have been reported to produce OTA, but these reports have apparently not been confirmed: *A. terreus* (sec. *Terrei*), *A. ustus* (sec. *Usti*; *A. versicolor* and *A. sydowii* (sec. *Versicolores*) (Ueno et al., cited in [24]).

In *Penicillium*, OTA was first detected in *P. viridicatum* [8]. However, Pitt later reassigned the ochratoxin-producing isolates to *P. verrucosum* [25]; in its current circumscription, *P. viridicatum* does not produce OTA. *P. chrysogenum* and *P. nordicum* have also been reported to produce OTA [26, 27].

In summary, OTA production is currently known in four sections of *Aspergillus* and in *Penicillium*, a curiously disjunct taxonomic distribution. As a result, it is unclear in some cases which fungi are responsible for ochratoxin contamination of foods. This problem is discussed below.

Variation in OTA production

There is tremendous intraspecific variation in OTA production. As with aflatoxins, this variation is a combination of phenotypic plasticity and genetic variation. In the *A. niger* group, for example, frequency of OTA production *in vitro* varied from $<2\%$ to $>90\%$, depending on the species and the study [24]. In *P. verrucosum* 74% of isolates from cereals in northern Europe produced OTA in culture [28]. Only 16% of AFLP haplotypes included more than one isolate, suggesting a high level of genetic variation.

*Most of the *Aspergillus* and *Penicillium* species discussed here lack a known sexual stage in their life cycles. Those that have sexual stages are usually classified in the genus that describes the sexual stage, in this case *Neopetromyces*. This dual system of nomenclature is required by the complicated life cycles of fungi. It is analogous to a scientist who uses her maiden name on publications, but her husband's last name for family business: she may be classified under two names which describe different aspects of her life. Both names are correct.

OTA has been detected in conidia. *Penicillium verrucosum* contained 0.4–0.7 pg OTA per conidium; conidia of *A. ochraceus* contained much less [29]. OTA has also been isolated from dust in cowsheds (presumably in the form of conidia) in concentrations up to 70 μg/kg (=ppb) [29]. OTA occurs in sclerotia, but in relatively low concentrations compared to aflatoxins in sclerotia of *A. flavus* [30].

As with aflatoxin contamination, insect damage may be a major cause of OTA contamination of coffee [31]. *A. ochraceus* was isolated from 5–17% of coffee berry borers (*Hypothenemus hampei*) emerging from coffee beans [32].

Which foods are contaminated by which fungi, and at what levels?

As the list of OTA-producing fungi has expanded, so has the list of foods which can be contaminated with ochratoxins. OTA was found in barley, wheat, rye, maize and rice starting in the 1960s [33]. More recently, OTA has been found in legumes, grapes, raisins and wines [24, 34], in nuts [Pitt and Hocking, cited in 35], in beers, in cacao and in spices [36]. OTA is also found in animal products, including cow's milk, and pork, particularly pork kidneys, liver and sausages [37, 38]. Coffee is discussed below. Since many of these have been identified only recently, it is likely that the list of OTA-contaminated foods will grow in the future.

The fungi responsible for OTA contamination vary from crop to crop and from place to place. The general rule, succinctly stated by John Pitt, is "Ochratoxin A...is produced by *Penicillium verrucosum* in cereal grains in cold climates, by *A. carbonarius* in grapes, wines and vine fruits, and by *A. ochraceus* sometimes in coffee beans" [35]. However, *P. verrucosum* has recently been found in cereals in warmer climates: Italy, Spain, France and Portugal [39].

As with aflatoxins, ochratoxin contamination of foods is highly variable. Some of the foods mentioned here rarely contain ochratoxin, or typically contain extremely low levels, or both. Variability in OTA production, combined with the large particle size of some of the foods it contaminates, complicates sampling strategies for detection of ochratoxins [40]. A few, highly contaminated grains or fruits can raise the level of a whole shipment above the allowed limit, if the limit is as low as 5 μg/kg. However, many sampling strategies are not designed to detect consistently these few, highly contaminated grains [40].

In tree nut orchards and figs in California, we found that *A. ochraceus* and *A. melleus* were the most common OTA-producing species, but no isolates produced OTA *in vitro* above the level of detection (10 μg/kg) [41]. Since 2002, the proportion of *A. ochraceus* and *A. melleus* isolates that produce OTA *in vitro* has varied from year to year [Palumbo and Baker, unpublished]. However, the fact that the most common OTA-producing species in tree nut orchards are dominated by atoxigenic isolates may explain why OTA contamination of tree nuts is rare in California [41]. On the other hand, *A. alliaceus* isolates from figs consistently produced OTA in vitro, up to 30,000 μg/kg. Presence of OTA in figs was correlated with presence of sporulating colonies of *A. alliaceus*, but not with presence of the *A. ochraceus* group or *Penicillium*. We have also found OTA-producing isolates of *A. lanosus* [13], *A. sclerotiorum* and *A. melleus* (unpublished) in tree nut orchards in California.

Average daily OTA intake was estimated at 0.7–4.6 ng/kg body weight, based on a typical European diet and typical levels of ochratoxins in various foods, with over half the OTA coming from cereals [42].

Which fungi cause OTA contamination of coffee, and at what levels?

There is a large body of literature documenting OTA contamination of green and processed coffee, and reporting isolation of OTA-producing fungi from coffee, starting in the 1970s [43–45]. Studies have concluded that *A. ochraceus*, *A. carbonarius* and *A. niger* may all be responsible, though most studies have focused on *A. ochraceus* [45]. Frisvad et al. [17] said that the most important OTA-producing species on coffee are *A. ochraceus*, *A. westerdijkiae* and *A. steynii*. Two new OTA-producing species in *Aspergillus* section *Nigri* were recently described from coffee [20].

Despite the large number of studies, we believe it is still not clear which species are most responsible for OTA contamination of coffee. Some

authors appear to have lumped other species in section *Circumdati* in *A. ochraceus sensu latu*, because they do not mention other species that common on coffee (e.g., *A. melleus*). Also, many of the *A. ochraceus* isolates from coffee should now be assigned to *A. westerdijkiae* and *A. steynii* [17]. Our data on isolates from Latin American coffee beans suggest that *A. ochraceus sensu strictu* rarely produces OTA, though *A. steynii* does (unpublished). Levels of OTA contamination, and probably the species that produce OTA, vary from region to region. Coffees from Africa generally have higher levels of OTA than coffees from Latin America or Asia [44].

OTA contamination of commercial, packaged coffees is very common, despite the fact that over half of OTA is destroyed during roasting [43, 46, 47]. For example, 80% of instant coffees sampled in the UK contained >0.1 μg/kg OTA, as did 85% of ground, roast coffees [48]. Instant coffees contained up to 8 μg/kg, which exceeds the current EU limit (5.0 μg/kg).

Biosynthesis of ochratoxins

The OTA biosynthetic pathway has been studied with ^{14}C-labelled precursors in *A. ochraceus* (now *A. steynii*, see [17]). The isocoumarin moiety is formed from acetate units via the pentaketide pathway, carboxylated, and then chlorinated by chloroperoxidase to form ochratoxin-α [49]. The final step, linkage through the carboxyl group to phenylalanine, is catalyzed by OTA synthetase. Ochratoxin B may be formed when chlorine concentrations are low, and to some extent by dechlorination of OTA [49].

Inhibition of OTA biosynthesis by various compounds and natural products has been studied, both as a tool to understand the pathway and as a strategy to prevent OTA contamination of foods. Alkaloids from *Piper longum*, turmeric and sesame inhibited OTA production *in vitro* [50]. However, effects of these alkaloids varied among three OTA-producing species (*A. alliaceus*, *A. auricomus* and *A. sclerotiorum*). For example, the alkaloid piperine significantly decreased OTA and OTB production in *A. sclerotiorum* (one isolate) and *A. alliaceus* (one isolate) but not in an isolate of A. *steynii*; in a second isolate of *A. alliaceus*, piperine decreased production of OTA but significantly increased production of OTB. These differences among isolates suggest biosynthesis of OTA is not identical in all fungi that produce it [50].

A polyketide synthase (*pks*) gene necessary for OTA biosynthesis in *A. ochraceus* was cloned by O'Callaghan et al. [51]. The polyketide synthase was only expressed on media on which OTA was produced, and mutants with insertions in the *pks* gene did not produce OTA. Since the polyketide genes responsible for aflatoxin production are clustered [52], genes for OTA may also be clustered. If this is true, the cloning of this *pks* gene should lead to the identification of other genes in the ochratoxin pathway [53].

As with aflatoxins and many other fungal secondary metabolites [54] the function of OTA for the fungus is unknown, though it has been shown to cause mortality and weight loss in insect larvae at concentrations of 2.5–25 ppm [55].

Levels of OTA in human tissues

Most people have detectable levels of OTA in the bloodstream (at least in certain countries), though usually at very low levels. OTA was detected in 100% of human blood samples (maximum 0.04 μg/L) and 58% of human milk samples (maximum 0.9 μg/L) in Norway [56]. Similar results have been reported in other studies. Blood plasma levels of OTA in Sweden ranged from 0.09 to 0.9 μg/l [56]. OTA was found in 58% of breast milk samples, but at levels about 10x lower than blood plasma levels. (This is the reverse of aflatoxins, where the highest levels in cows are found in milk as aflatoxin M_1 [57]). OTA was detected in 21% of breast milk samples in a Norwegian study, at levels up to 1.8 μg/l; OTA was correlated with consumption of liver paté, cakes and juices [58]. Other studies have found OTA levels as high as 6.6 μg/l in breast milk (see [29, 56]). These levels are alarming because they suggest that some infants are exposed to levels above the recommended maximum dose of 5 ng/kg body weight per day.

OTA levels in blood of nephropathy patients are higher than those in healthy controls. In a Tunisian study, both blood and food samples from nephropathy patients had significantly higher OTA levels than healthy controls (see [59]).

Patients had blood OTA levels up to 1136 μg/l, and foods they provided had up to 46,000 μg/kg. Interpretation of these results implies an untested assumption: that patients with high levels of OTA in their diets when tested also had high levels in the past, when the kidney damage occurred. In a Taiwanese study, excretion of OTA in urine of diabetes mellitus patients was significantly higher than in control patients, and patients with other types of nephropathy [60]. Also, OTA excretion in urine was positively and significantly correlated with excretion of urine protein (a symptom of nephropathy). These studies show correlation rather than causality, but taken together they suggest that exposure to high OTA levels is associated with kidney damage.

Most studies on ochratoxin levels in human tissues have been done in Europe, motivated by concern about ochratoxin contamination of cereals. It is possible that levels are lower in other regions, although there are other sources of OTA in the diet. For example, a study of breast milk in Brazil found that most samples were negative, and the maximum detected was 0.02 μg/l [61].

Toxicity in animals and humans

Ochratoxin A is classified as a possible human carcinogen (group 2B) [1, 59]. However, as with aflatoxins, it is often not clear to what extent animal studies are applicable to toxicity in humans. Interpretation of epidemiological studies is often difficult because of other factors that cannot be controlled, including co-occurrence with other mycotoxins that may have synergistic effects [62]. OTA has been reported to be nephrotoxic, immunosupressive, carcinogenic and teratogenic in animal studies [10, 53]. Oral LD_{50} values are 20 mg/kg for young rats and 3.6 mg/kg for chicks [35].

There are several mechanisms for OTA toxicity at the cellular level. OTA is a competitor of phenylalanine-tRNA ligase, thereby inhibiting protein synthesis. Presence of phenylalanine or Aspartame (an analogue of phenylalanine) decreases toxicity by competition with OTA [42, 63]. Other enzymes are also affected by exposure to OTA [1, 53]. Other mechanisms include formation of DNA adducts, apoptosis, interference with the cytoskeleton, lipid peroxidation and inhibition of mitochondrial respiration [1, 53].

OTA toxicity is most acute in the kidney [58]. Experimental exposure to OTA causes various types of kidney lesions in animal models [1]. It can cause serious kidney diseases in pigs in Scandinavia, and also in poultry [1, 53].

OTA elimination is slower in humans than in all other species tested, providing more contact time for damage to occur [1]. OTA is thought to be the cause of two chronic diseases, Balkan Endemic Nephropathy (BEN) and Chronic Interstitial Nephropathy (in North Africa), and of urothelial tumors in humans [53]. DNA adducts from patients in the Balkans provide further evidence for the involvement of OTA in BEN.

A link between OTA exposure early in life and testicular cancer has been hypothesized, based on epidemiological associations [64]. OTA exposure is high in Denmark, probably due to high consumption of rye and pork, and testicular cancer is high as well. Both are higher in northern Europe than in southern and central Europe, and have apparently increased over the past 50 years.

So how hazardous is OTA to human health? Recent studies have reached varying conclusions. The EU Scientific Committee on Food recommended a maximum of 5 ng/kg body weight/day [65]. However, other studies have found "...that there are no health risks associated with the dietary intake of OTA in the Netherlands;" (Dutch National Institute of Public Health and the Environment, 2003, cited from [66]); and that "...no cases of acute intoxication in humans have been reported" [FAO/WHO Joint Expert Committee on Food Additives and Contaminants, 2001, cited from 30]. Nevertheless, the evidence mentioned above strongly suggests that OTA causes acute and chronic disease in certain places and circumstances.

Regulation of ochratoxins in foods

The European Union (EU) limits ochratoxin A in imported foods, with a maximum of 5 μg/kg (= ppb) in raw cereal grains, 3 μg/kg in processed cereal foods, and 10 μg/kg in dried vine fruits (raisins) [65]. As of April 2005, the EU imposed limits for ochratoxins in wine, grape juice and coffee. The limits are 2.0 μg /kg for wine and grape juice, 5.0 μg/kg for roasted coffee, and 10.0 μg/kg for instant coffee [66]. Limits for green coffee

beans were not imposed, but this policy is to be reviewed in 2006. However, a number of European nations have already set limits for ochratoxin A in green coffee beans; the most stringent is Italy, at 8 μg/kg.

Of particular concern are the limits for ochratoxins in coffee. Coffee is one of the world's most valuable crops, and its export is crucial to the economy of many developing countries. Coffee beans are often processed outdoors or in semienclosed facilities, making it difficult to control moisture during processing and storage. According to projections by the European Coffee Federation, a maximum limit of 5 μg/kg on green coffee could mean an average rejection rate of traded lots of around 7%, and up to 18% for some African producers [47]. This could have a serious impact on the economies of the exporting countries.

In USA, in contrast, the FDA has not set advisory limits or action levels for ochratoxins in any commodity.

Future directions

Key questions in the ochratoxin story are still not completely answered, especially in the areas of OTA biosynthesis, OTA contamination of crops, and toxicity and epidemiology of OTA.

Do all ochratoxin-producing fungi have the same biosynthetic pathway? Why are so many isolates atoxigenic? Can knowledge of the pathway and its regulation be used to control OTA contamination of crops? The genetics of aflatoxin production has advanced rapidly [6, 52] and provides tools applicable to OTA, so we expect that these questions will be answered over the next few years.

How can ochratoxin contamination of crops be prevented? Could a biocompetition strategy using atoxigenic isolates reduce OTA contamination of crops? Field application of nontoxigenic isolates of *A. flavus* has been very successful in reducing aflatoxin contamination of cottonseed, corn and peanuts [67]. A similar strategy could be applicable to OTA contamination, especially for high-value crops like coffee and wine grapes.

How serious a threat does OTA pose to human health, and at what levels? Other factors may influence the impact of OTA on human health. Diabetes affected 4% of the world population in 1995, and is projected to rise to 5.4% in 2025 [68]. In USA diabetes cases more than doubled from 1980 to 2003, and rates are much higher among blacks and latinos than among whites. The apparent interactions between OTA and diabetes mellitus may make OTA exposure an important concern for this sector of the population. The relationship between OTA and diabetes is still unclear. However, in terms of public health implications it may reflect the relationship between aflatoxins and hepatitis B and C: they are co-carcinogens, and the growing number of hepatitis B and C cases means that threat of liver cancer from aflatoxins is growing as well [62].

What effects would the proposed EU limits on OTA in green coffee have on economies of coffee-producing countries? What effects would be felt by growers in the USA if the FDA sets action levels for OTA in foods? The best way to answer these questions is to look at the costs of aflatoxin regulation [57, 69].

According to UN Secretary Kofi Annan, "...a World Bank study has calculated that the European Union regulation on aflatoxins costs Africa $750 million each year in exports of cereals, dried fruit and nuts. And what does it achieve? It may possibly save the life of one citizen of the European Union every 2 years ... Surely a more reasonable balance can be found." (cited from [69]). Henry et al. suggested that the most cost-effective strategy for managing aflatoxins is to vaccinate populations against hepatitis, rather than imposing ever more stringent limits on aflatoxins in foods [62].

Annan's statement may also apply to ochratoxins, especially in coffee. Also, it illustrates the difficulty of balancing potential health benefits (from strict limits on ochratoxins) with potential economic losses (caused by those strict limits). Furthermore, limits on ochratoxins content in foods imported by developed countries may increase exposure to ochratoxins for people in developing countries; what cannot be exported may be consumed domestically. Achieving the right balance is complicated by the tremendous variation in ochratoxin production and contamination among organisms and crops, and the difficulty of extrapolating toxicity data from animal studies to humans.

We suggest that the debate over regulation of ochratoxins is potentially even greater than that

for aflatoxins. The range of commodities they are known to contaminate is greater (and still growing); they include commodities like coffee and wine, which are vital to the economies and self-images of many countries. Also, relative to aflatoxins, ochratoxins are more likely to have serious health impacts in developed countries, where most such regulations are established and enforced [1].

Given these uncertainties and trends, it is entirely possible that ten years from now, ochratoxins will have overshadowed aflatoxins in terms of public interest and debate.

Acknowledgments

We thank the NIH-SCORE and NOAA-Sea Grant programs for financial support, Jeffrey D. Palumbo (USDA ARS WRRC) for unpublished data, and Noreen E. Mahoney (USDA ARS WRRC) for ochratoxin analysis.

References

1. Størmer FC. Ochratoxin A – a mycotoxin of concern. In: Bhatnagar D, Lillehoj EB, Arora DK, eds. Handbook of Applied Mycology, Vol. 5: Mycotoxins in Ecological Systems, Marcel Dekker, Inc, New York, 1992: 403–432.
2. Hooper J. Polluted pasta causes toxin alarm in Italy. Guardian Unlimited 1/12/2006. http://www.guardian.co.uk/food/Story/0,1684524,00.html.
3. Matossian MK. Mold poisoning and population growth in England and France, 1750–1850. J Econ Hist 1984; 44: 669–686.
4. Di Paolo N, Guarnieri A, Loi F, Sacchi G, Mangiarotti AM, Di Paolo M. Acute renal failure from inhalation of mycotoxins. Nephron 1993; 64: 621–625.
5. van der Merwe KJ, Steyn PS, Fourie L. Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus* Wilh. Nature 1965; 205: 1112–1113.
6. Bennett JW, Klich M. Mycotoxins. Clin Microbiol Rev 2003; 16: 497–516.
7. Lai M, Semeniuk G, Hesseltine CW. Conditions for production of ochratoxin A by *Aspergillus* species in synthetic medium. Appl Microbiol 1970; 19: 542–544.
8. Ciegler A, Fennell DI, Sansing GA, Detroy RW, Bennett GA. Mycotoxin-producing strains of *Penicillium viridicatum*: Classification into subgroups. Appl Microbiol 1973; 26: 271–278.
9. Hesseltine CW, Vandegraft EE, Fennell DI, Smith ML, Shotwell OL. Aspergilli as ochratoxin producers. Mycologia 1972; 64: 539–550.
10. Tsubouchi H, Terada H, Yamamoto K, Hisada K, Sakabe Y. Caffeine degradation and increased ochratoxin production by toxigenic strains of *Aspergillus ochraceus* isolated from green coffee beans. Mycopathologia 1995; 90: 181–186.
11. Varga J, Kevei É, Rinyu E, Téren J, Kozakiewicz Z. Ochratoxin production by *Aspergillus* species. Appl Environ Microbiol 1996; 62: 4461–4464.
12. Frisvad JC, Samson RA. *Neopetromyces* gen. nov. and an overview of teleomorphs of *Aspergillus* subg. *Circumdati*. Stud Mycol 2000; 45: 201–207.
13. Baker JL, Bayman P, Mahoney NE, Klich MA, Palumbo JD, Campbell BC. Ochratoxigenic *Aspergillus lanosus* and *A. alliaceus* isolates from California tree nut orchards. Proceedings of the 3rd Fungal Genomics, 4th Fumonisin, and 16th Aflatoxin Elimination Workshops, Savannah, Georgia, 2003 [Poster].
14. Peterson SW. Phylogenetic relationships in *Aspergillus* based on rDNA sequence analysis. In: Samson RA, Pitt JI, eds. Classification of *Penicillium*, *Aspergillus*: Integration of Modern Taxonomic Methods, Harwood Publishers Reading UK, 2000: 323–356.
15. Varga J, Kevei É, Tóth B, Kozakiewicz Z, Hoekstra RF. Molecular analysis of variability within the toxigenic *Aspergillus ochraceus* species. Can J Microbiol 2000; 46: 593–599.
16. Varga J, Tóth B, Rigó K, Téren J, Hoekstra RF, Kozakiewicz Z. Phylogenetic analysis of *Aspergillus* section *Circumdati* based on sequences of the internal transcribed spacer regions and the 5.8S rRNA gene. Fungal Genet Biol 2000; 30: 71–80.
17. Frisvad JC, Frank JM, Houbraken J, Kuijpers AFA. New ochratoxin producing species of *Aspergillus* section Circumdati. Stud Mycol 2004; 50: 23–43.
18. Abarca ML, Bragulat MR, Sastellá G, Cabañes FJ. Ochratoxin A production by strains of *Aspergillus niger* var. *niger*. Appl Environ Microbiol 1994; 60: 2650–2652.
19. Téren J, Varga J, Hamari Z, Rinyu E, Kevei F. Immunochemical detection of ochratoxin A in black *Aspergillus* strains. Mycopathologia 1996; 134: 171–176.
20. Samson RA, Houbraken JAMP, Kuijpers AFA, Frank JM, Frisvad JC. New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. Stud Mycol 2004; 50: 45–61.
21. Schuster E, Dunn-Coleman N, Frisvad J, Dijck Pvan. On the safety of *Aspergillus niger* – a review. Appl Microbiol Biotechnol 2002; 59: 426–435.
22. Blank G, Nwoko U, Frohlich A, Marquardt R. Ochratoxin A production in relation to the growth morphology of *Aspergillus alutaceus*. Lebensm Wiss Technol 1998; 31: 210–214.
23. Klich MA. Identification of Common *Aspergillus* Species. Utrecht, Netherlands: Centraalbureau voor Schimmelcultures, 2002.
24. Abarca ML, Accensi F, Bragulat MR, Cabañes FJ. Current importance of ochratoxin A-producing *Aspergillus* spp. J Food Protect 2001; 64: 903–906.
25. Pitt JI. *Penicillium viridicatum*, *Penicillium verrucosum*, and production of ochratoxin A. Appl Environ Microbiol 1987; 53: 266–269.
26. Land CJ, Hult K. Mycotoxin production by some wood-associated *Penicillium* spp. Lett Appl Microbiol 1987; 4: 41–44.

27. Mills JT, Abramson D. Ochratoxigenic potential of *Penicillium* spp. isolated from stored rapeseed and cereals in western Canada. Can J Plant Pathol 1982; 4: 37–41.
28. Frisvad JC, Lund F, Elmholt S. Ochratoxin A producing *Penicillium verrucosum* isolates from cereals reveal large AFLP fingerprinting variability. J Appl Microbiol 2005; 98: 684–692.
29. Skaug MA, Helland I, Solvoll K, Saugstad OD. Presence of ochratoxin A in human milk in relation to dietary intake. Food Addit Contam 2001; 18: 321–327.
30. Paster N, Lisker N, Chet I. Accumulation of ochratoxin A in sclerotia of *Aspergillus ochraceus*. Can J Bot 1985; 63: 661–662.
31. Bucheli P, Kanchanomai C, Meyer I, Pittet A. Development of ochratoxin A during robusta (*Coffea canephora*) coffee cherry drying. J Agric Food Chem 2000; 48: 1358–1362.
32. Vega FE, Mercadier G. Insects, coffee and ochratoxin A. Fla Entomol 1998; 81: 543–544.
33. Shotwell LL, Hesseltine CW, Goulden ML. Ochratoxin A occurrence as natural contaminant of a corn sample. Appl Microbiol 1969; 17: 765–766.
34. Zimmerli B, Dick R. Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. Food Addit Contam 1996; 13: 655–668.
35. Pitt JI. Toxigenic fungi: Which are important? Med Mycol 2000; 38S: 17–22.
36. Aziz NH, Youssef YA, El-Fouly MZ, Moussa LA. Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. Bot Bull Acad Sinica (Taiwan) 1998; 39: 279–285.
37. Jorgensen K. Survey of pork, poultry, coffee, beer and pulses for ochratoxin. Food Addit Contam 1998; 15: 550–554.
38. Kuiper-Goodman T, Scott PM. Risk assessment of the mycotoxin ochratoxin A. Biomed Environ Sci 1989; 2: 179–248.
39. Olsen M, Jonsson N, Magan N, Banks J, Fanelli C, Rizzo A, Haikara A, Dobson A, Frisvad J, Holmes S, Pajunen E, Persson SJ, Jonsson A. Prevention of Ochratoxin A in Cereals http://www.slv.se/upload/dokument/fou/MIB/OTA%20Prev%20Handout.pdf.
40. Campbell BC, Molyneux RJ, Schatzki TF. Current research on reducing pre- and post-harvest aflatoxin contamination of U.S. tree nuts. J Toxicol Toxin Rev 2003; 22: 225–266.
41. Bayman P, Baker JL, Doster MA, Michailides TJ, Mahoney NE. Ochratoxin production by the *Aspergillus ochraceus* group and *A. alliaceus*. Appl Environ Microbiol 2002; 68: 2326–2329.
42. Walker R. Mycotoxins of growing interest. Third Joint FAO/WHO/UNEP International Conference on Mycotoxins, Tunis, 1999. http://www.fao.org/es/ESN/ food/ pdf/ mycotoxins_report_en.pdf.
43. Levi CP, Trenk HL, Mohr HK. Study of the occurrence of ochratoxin A in green coffee beans. J Assoc Off Anal Chem 1974; 57: 866–870.
44. Romani S, Sacchetti G, Chaves López C, Pinnavaia GG, Dalla Rosa M. Screening on the occurrence of ochratoxin A in green coffee beans of different origins and types. J Agric Food Chem 2000; 48: 3616–3619.
45. Urbano GR, Taniwaki MH, Leitão MFF, Vicentini MC. Occurrence of ochratoxin A-producing fungi in raw Brazilian coffee. J Food Prot 2001; 64: 1226–1230.
46. Blanc M, Pittet A, Munoz-Box R, Viani R. Behavior of ochratoxin A during green coffee roasting and soluble coffee manufacture. J Ag Food Chem 1998; 46: 673–675.
47. International Coffee Organization (2005). OTA risk management: guidelines for green coffee buying. http://www.ico.org/documents/ed1939e.pdf.
48. Patel S, Hazel CM, Winterton AGM, Gleadle AE. Survey of ochratoxin A in UK retail coffees. Food Addit Contam 1997; 14: 217–222.
49. Harris JP, Mantle PG. Biosynthesis of ochratoxins by *Aspergillus ochraceus*. Phytochemistry 2001; 58: 709–716.
50. Lee SE, Bayman P, Baker JL, Campbell BC. Inhibition of ochratoxin biosynthesis by naturally occurring alkaloids. [in preparation].
51. O'Callaghan J, Caddick MX, Dobson ADW. A polyketide synthase gene required for ochratoxin A biosynthesis in *Aspergillus ochraceus*. Microbiology 2003; 149: 3485–3491.
52. Yu J, Chang P, Ehrlich K, Cary JW, Bhatnagar D, Cleveland TE, Payne GA, Linz JE, Woloshuk CP, Bennett JW. The clustered pathway genes in aflatoxin biosynthesis. Appl Environ Microbiol 2004; 70: 1253–1262.
53. O'Brien E, Dietrich DR. Ochratoxin A: The continuing enigma. Crit Rev Toxicol 2005; 35(1): 33–60.
54. Bennett JW. From molecular genetics and secondary metabolism to molecular metabolites and secondary genetics. Can J Bot 1995; 73: S917–S924.
55. Dowd PF. Toxicity of naturally occurring levels of the *Penicillium* mycotoxins citrinin, ochratoxin A, and penicillic acid to the corn earworm, *Heliothis zea*, and the fall army worm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environ Entomol 1989; 18: 24–29.
56. Breitholtz-Emanuelsson A, Olsen M, Oskarsson A, Palminger I, Hult K. Ochratoxin A in cow's milk and in human milk with corresponding human blood samples. J Assoc Off Anal Chem 1993; 76: 842–846.
57. Robens J, Cardwell K. The costs of mycotoxin management to the USA: Management of aflatoxins in the United States. J Toxicol Toxin Rev 2003; 22: 139–152.
58. Simon P. Ochratoxin and kidney disease in the human. J Toxicol Tox Rev 1996; 15: 239–249.
59. Codex Alimentarius Commission. Position paper on ochratoxin A. Food and Agriculture Organization of the United Nations, Rome, Italy, 1998. http://www.who.int/fsf/ Chemicalcontaminants /ochratoxinpp99_14.pdf.
60. Hsieh MF, Chiu HY, Lin-Tan DT, Lin JL. Does human ochratoxin A aggravate proteinuria in patients with chronic renal disease? Ren Fail 2004; 26: 311–316.
61. Navas SA, Sabino M, Rodriguez-Amaya DB. Aflatoxin M1 and ochratoxin A in a human milk bank in the city of São Paulo, Brazil. Food Addit Contam 2005; 22: 457–462.
62. Henry SH, Bosch FX, Troxell TC, Bolger PM. Reducing liver cancer–global control of aflatoxin. Science 1999; 286: 2453–2454.
63. Creppy EE, Baudrimont I, Belmadani A, Betbeder AM. Aspartame as a preventive agent of chronic toxic effects of ochratoxin A in experimental animals. J Toxicol Toxin Rev 1996; 15: 207–221.

64. Schwartz GG. Hypothesis: Does ochratoxin A cause testicular cancer?. Cancer Causes Control 2002; 13: 91–100.
65. FAO. Worldwide regulations for mycotoxins in food and feed in 2003. 2004. http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/007/y5499e/y5499e0f.htm.
66. FAO. Reducing ochratoxin A in coffee. 2005. http://www.coffee-ota.org/proj_background.asp.
67. Ehrlich KC, Cotty PJ. An isolate of *Aspergillus flavus* used to reduce aflatoxin contamination in cottonseed has a defective polyketide synthase gene. Appl Microbiol Biotechnol 2004; 65: 473–478.
68. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995–2025. Diabetes Care 1998; 21: 1414–1431.
69. Cardwell KF, Desjardins A, Henry SH, Munkvold G, Robens J. Mycotoxins: the cost of achieving food security and food quality. APSnet Feature Story, 2001. http://www.apsnet.org/online/feature/mycotoxin/.

Address for correspondence: Paul Bayman, Depto. de Biología Universidad de Puerto Rico-Río Piedras, PO Box 23360, San Juan, PR, 00931 , USA
E-mail: pbayman@upracd.upr.clu.edu